
Protocol Section:	Embryo Cryopreservation	Policy No:	P-CMMR1-ECD-01
Protocol Subject:	Modified Rapid Embryo Thawing Protocol D for ENU and Non-ENU Mice Embryos at Eight-Cell Stage	Effective Date:	
		Date Reviewed:	24 October 2004
		Date Revised:	17 May 2006

Objective

To thaw embryos stored in plastic straw that was frozen according to the Canadian Mutant Mouse Repository's (CMMR) Modified Rapid Embryo Freezing Protocol D, which based on the procedure described by A. Trounson *et al. Fertil. Steril.*, 48, 843-850 with some modification.

Materials

- Liquid nitrogen dewar
- 37 degrees Celsius water bath
- Scissors
- Kimwipes
- Timer
- Forceps
- Mouth Pipette
- 1cc syringe, load up with 0.25M sucrose in dPBS
- 100 x 15mm petri-dish
- Modified Dulbecco's Phosphate Buffered saline (dPBS) containing 3mg/ml of BSA –(Purchase from commercial vendors or refer to CMMR's Modified Dulbecco's Phosphate Buffered Saline protocol for house-made recipe)
- 0.25M Sucrose in dPBS; dissolve 860mg of sucrose in 10ml of dPBS (as described above)

Procedure

1. Transfer straw containing embryos from the liquid nitrogen dry shipper into a smaller dewar with liquid nitrogen.
2. Using forceps, plunge the straw into a 37°C water bath in one quick motion until the media in straw turn from frosty to clear. (ice disappears)
3. Remove straw from water bath and wipe dry gently with kimwipe
4. Holding the straw in horizontal position, carefully cut off the heat sealed end and another cut just below the cotton plug. (Therefore the cotton plug will be cut away)
5. Insert the straw from the end where the cotton plug was cut off into a 1cc syringe loaded with 0.25M sucrose in dPBS (refer to materials section)
6. Flush out the embryos in straw using 1ml of sucrose solution in the syringe onto a 100 x 15mm petri-dish.
7. Incubate in room temperature for 10 minutes as the cryoprotectants and water leave the cell under osmotic influence of the sucrose solution.
8. Transfer embryos into a fresh drop of dPBS (500ul) using a mouth pipette and incubate at room temperature for another 10 minutes.
9. Wash the embryos with 10 fresh drops (100ul each) of dPBS. Embryos are best to be transferred into a 2.5-

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3dpc pseudopregnant recipient as soon as possible.

Issued by Lab Manager: _____ **Date:** _____

Approved by Facility Management: _____ **Date:** _____